CLEANING UP WITH GENOMICS: APPLYING MOLECULAR BIOLOGY TO BIOREMEDIATION

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Bioremediation has the potential to restore contaminated environments inexpensively yet effectively, but a lack of information about the factors controlling the growth and metabolism of microorganisms in polluted environments often limits its implementation. However, rapid advances in the understanding of bioremediation are on the horizon. Researchers now have the ability to culture microorganisms that are important in bioremediation and can evaluate their physiology using a combination of genome-enabled experimental and modelling techniques. In addition, new environmental genomic techniques offer the possibility for similar studies on as-yet-uncultured organisms. Combining models that can predict the activity of microorganisms that are involved in bioremediation with existing geochemical and hydrological models should transform bioremediation from a largely empirical practice into a science.

> "And you know, it makes me wonder what's going on down under the ground"
>
> *David Crosby*

Microorganisms can aid environmental restoration by oxidizing, binding, immobilizing, volatilizing or otherwise transforming contaminants. There is significant interest in such microbially mediated bioremediation because it promises to be simpler, cheaper and more environmentally friendly than the more commonly used 'muck, suck and truck' non-biological options, in which contaminants are merely dug or pumped up and then transported elsewhere. However, in general, the promise of bioremediation has yet to be realized — bioremediation strategies that are successful in one location might not work in another, and microbial processes that remediate contaminants in laboratory incubations might not function well in the field. The reasons for such failures are often not obvious and many practitioners are therefore unwilling to risk the bioremediation option. Another factor limiting the implementation of bioremediation is that, unlike the concepts of excavation and disposal, which are easy to grasp, the mechanisms controlling the growth and activity of microorganisms in contaminated environments are not well understood, even by the most knowledgeable microbiologists.

Ideally, bioremediation strategies would be designed based on knowledge of: the microorganisms that are present in the contaminated environments; their metabolic capabilities; and how they respond to changes in environmental conditions. Unfortunately, in practice, much of the required information is not readily available and the use of microorganisms in bioremediation is highly empirical rather than knowledge-based. This contrasts with a much more mature understanding of the relevant chemical and physical processes in contaminated environments, which can be encoded in highly sophisticated geochemical and hydrological models (BOX 1). If comparable models to predict the activity of microorganisms during bioremediation could be developed, then the implementation of bioremediation strategies might one day become as readily comprehensible as 'muck, suck and truck'.

Although the science of bioremediation is still far from this goal at present, it now seems attainable.

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Box 1 | Modelling contaminant transport

Modelling the transport and fate of contaminants in dynamic aqueous environments, such as aquifers, requires the ability to describe not only the rate and direction of water movement, but also the transformations of contaminants and other chemical species that might influence contaminant degradation or transport. If it were not for biological reactions, this might be readily feasible using existing geochemical and hydrological models[82-84]. The path of groundwater flow can be predicted from relatively simple measurements of water levels in specialized wells called PIEZOMETERS[85]. From a given point the water moves from areas with higher water levels to areas of lower water level. Rates of groundwater flow can be estimated from the time it takes a relatively unreactive tracer — such as bromide — to move between wells, or rates can be inferred with less-direct methods. The geochemical speciation of contaminants and other aquifer constituents into various dissolved and mineral phases can be predicted with sophisticated geochemical models[83,84]. Constants for the adsorption of contaminants to aquifer material can readily be measured in laboratory incubations and incorporated into models to predict the influence of contaminant partitioning between dissolved and adsorbed phases on migration of the contaminant within the aquifer. Coupling models for the concentrations of constituents in the water and the rate of groundwater flow results in a model for transport of these solutes. An example in which such solute-transport models might accurately predict transport of a contaminant is the migration of uranium through an organic-poor, sandy aquifer, in which there is little microbial activity to influence metal mobility[86]. However, in most contaminated environments the activity of microorganisms influences the fate of inorganic as well as organic contaminants, either owing to direct enzymatically catalysed transformations of the contaminants or by altering the chemistry of the environment in such a way as to affect contaminant solubility. In contrast to the relatively simple mathematical expressions that can describe the geochemical equilibration of a given element in waters of various compositions, or the flow of water in different subsurface matrices, mathematical descriptions of the metabolism of microorganisms under various environmental conditions will be very complex. At present, models that include microbially catalysed reactions rely on kinetic constants to describe the rates of microbial metabolism. These rate constants are typically fixed numbers that are derived from laboratory studies or are estimated for the environment under study by varying the constants until the model output fits the available geochemical data. This type of modelling of microbial processes has little predictive value over a diversity of contaminated sites.

The advent of high-throughput methods for DNA sequencing and the analysis of gene expression and function, as well as advances in the modelling of microbial metabolism, are revolutionizing the study of environmental microbiology. The purpose of this review is to outline how genome-enabled studies of microbial physiology and ecology are being applied to the field of bioremediation, and to anticipate additional applications of genomics that are likely in the near future. This review will be restricted primarily to *in situ* bioremediation in SUBSURFACE ENVIRONMENTS, because the inaccessibility, and often large areas, of subsurface contamination often makes bioremediation the most attractive strategy for the restoration of such environments. The potential role of genetically engineered microorganisms will not be discussed because the use of genetically engineered microorganisms for large-scale *in situ* bioremediation has yet to be adopted as an acceptable option by many regulatory agencies.

A general overview of bioremediation

Microorganisms have the capacity to remove many contaminants from the environment by a diversity of enzymatic processes. Extensive lists of microorganisms that carry out bioremediation reactions are available[1,2]. One common type of bioremediation is the oxidation of toxic, organic contaminants to non-toxic products, often carbon dioxide. Oxygen is the most commonly considered electron acceptor for microbial respiration, and the aerobic degradation of an extensive range of organic contaminants, from aromatic hydrocarbons, such as benzene, to XENOBIOTICS, such as pesticides, has been studied in detail[2]. Although a wide phylogenetic diversity of microorganisms is capable of aerobic contaminant degradation[1,2], *Pseudomonas* species and closely related organisms have been the most intensively investigated owing to their ability to degrade so many different contaminants.

Many polluted environments — such as AQUIFERS, aquatic sediments and submerged soils — are often ANOXIC and it is becoming increasingly apparent that microorganisms can anaerobically oxidize many contaminants with alternative electron acceptors, such as nitrate, sulphate and Fe(III) oxides[1,3], and possibly electrodes[4]. The use of these electron acceptors is often segregated into distinct zones (FIG. 1), which are based on electron-acceptor availability and the competition of different respiratory types of microorganisms for electron donors[1]. For example, Fe(III) is often the most abundant potential electron acceptor for the oxidation of organic matter in subsurface environments[5], and enhancing the availability of Fe(III) for microbial reduction can greatly stimulate the anaerobic degradation of organic contaminants[6,7]. As detailed below, *Geobacter* species, which can oxidize organic compounds with the reduction of Fe(III)[8], are highly enriched in subsurface environments in which organic contaminants are oxidized with the reduction of Fe(III). Sulphate is a particularly important electron acceptor for the anaerobic degradation of contaminants in marine environments[9] owing to the high concentrations of sulphate in seawater, and the addition of sulphate to groundwater can greatly accelerate contaminant degradation in aquifers[10]. Sulphate-reducing microorganisms, such as *Desulfobacula* and *Desulfobacterium* species, can oxidize hydrocarbons with sulphate as the electron acceptor[11].

Some contaminants serve as electron acceptors rather than electron donors in bioremediation reactions. For example, one of the most important types of bioremediation is reductive dechlorination, in which microorganisms remove chlorines from contaminants, such as chlorinated solvents and polychlorinated biphenyls, by using these compounds as electron acceptors in respiration[12]. Many organisms capable of dehalogenation are known[13] but, as discussed below, *Dehalococcoides* species seem to be particularly important in catalysing this reaction in contaminated subsurface environments. Some microorganisms can reduce inorganic contaminants, such as nitrate and perchlorate, converting them to innocuous products[1,14]. Metals are another class of contaminants that can serve as electron acceptors in microbial respiration[1]. Although the reduction of metals does not destroy them, it often changes their solubility. For example, *Geobacter* species can use uranium (U) as an electron acceptor; reducing the soluble, oxidized form

SUBSURFACE ENVIRONMENT
An environment that is below the land surface.

XENOBIOTIC
A chemical that is only man-made, and is otherwise not found in the environment.

AQUIFER
A water-saturated subsurface environment.

ANOXIC
A state lacking in oxygen.

PIEZOMETER
A well in an aquifer for determining water levels to estimate the direction of groundwater flow.

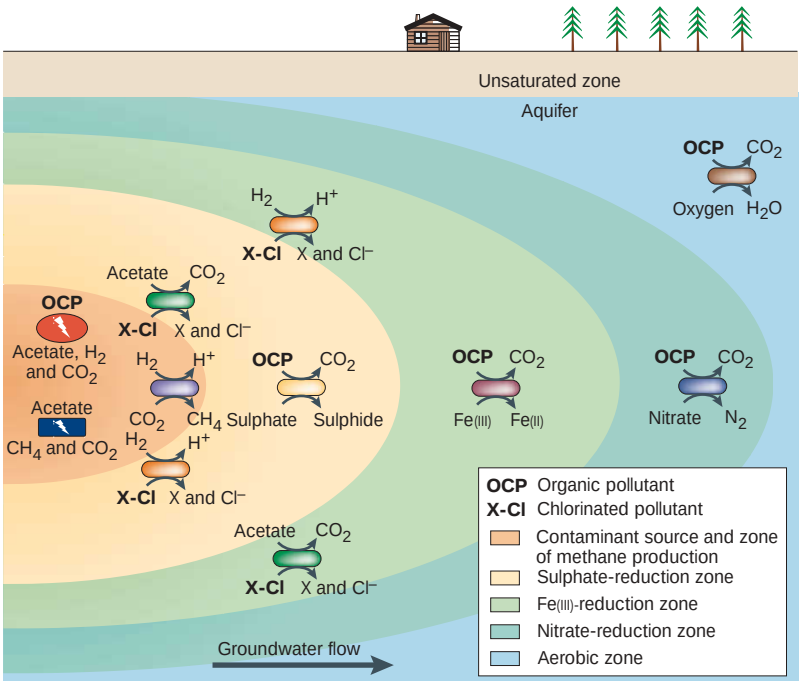

Figure 1 | **Typical bioremediation reactions for oxidizable, organic contaminants and chlorinated solvents in contaminated aquifers.** Generally, there are distinct zones in which different degradative processes predominate. At the source of contamination, such as the leachate emanating from a landfill, methane production often predominates. In this zone microorganisms convert organic contaminants to simpler molecules, such as acetate and hydrogen, which methane-producing microorganisms convert to methane. In other zones, organic contaminants are oxidized to carbon dioxide with the reduction of sulphate, Fe(III), nitrate or oxygen. Chlorinated contaminants, which are not easily oxidized, undergo reductive dechlorination in the methanogenic, sulphate-reduction or Fe(III)-reduction zones.

of uranium, U(VI), to the insoluble form, U(IV)[15], and stimulating the growth of *Geobacter* in uranium-contaminated subsurface environments. This precipitates the uranium from contaminated groundwater, thereby preventing its further spread[16].

Microbial removal of contaminants from the environment often takes place without human intervention. This has been termed intrinsic bioremediation[17]. Relying on intrinsic bioremediation is increasingly the bioremediation option of choice if it can be shown that the contamination does not pose an immediate health threat and it remains localized. If the rate of intrinsic bioremediation is too slow, then environmental conditions can be manipulated to stimulate the activity of microorganisms that can degrade or immobilize the contaminants of concern. Engineered bioremediation strategies include: the addition of electron donors or acceptors that will stimulate the growth or metabolism of microorganisms that are involved in the bioremediation processes; the addition of nutrients that limit the growth or activity of the microorganisms; and amendments to microorganisms with desired bioremediation capabilities.

Pre-genomics approaches to bioremediation

Non-molecular techniques. At present, most applied microbiological investigations of bioremediation processes make use of the 'treatability study', in which samples of the contaminated environment are incubated in the laboratory and the rates of contaminant degradation or immobilization are documented[18]. Such studies provide an estimate of the potential metabolic activity of the microbial community, but give little insight into the microorganisms that are responsible for the bioremediation, or why particular amendments that can be evaluated for engineered bioremediation applications do, or do not, stimulate activity.

When bioremediation processes are researched in more detail, attempts are generally made to isolate the organisms responsible[18]. The isolation and characterization of pure cultures has been, and will continue to be, crucial for the development and interpretation of molecular analyses in microbial ecology (FIG. 2). The recovery of isolates that are representative of the microorganisms responsible for the bioremediation process can be invaluable because, as outlined below, studying these isolates provides the opportunity to investigate not only their biodegradation reactions, but also other aspects of their physiology that are likely to control their growth and activity in contaminated environments. However, before the application of molecular techniques to bioremediation, it was uncertain whether the isolated organisms were important in bioremediation *in situ*, or whether they were 'weeds' that grew rapidly in the laboratory but were not the primary organisms responsible for the reaction of interest in the environment.

The 16S rRNA approach: what microorganisms are there? A significant advance in the field of microbial ecology was the finding that the sequences of highly conserved genes that are found in all microorganisms, most notably the 16S rRNA genes, could provide a phylogenetic characterization of the microorganisms that comprise microbial communities[19,20]. This was a boon to the field of bioremediation because it meant that by analysing 16S rRNA sequences in contaminated environments, it was possible to determine definitively the phylogenetic placement of the microorganisms that are associated with bioremediation processes[18,21].

One of the surprises from the application of the 16S rRNA approach to bioremediation has been the finding that, in some instances, microorganisms that predominate during bioremediation are closely related to organisms that can be cultured from subsurface environments[3]. This contrasts with the general dilemma in environmental microbiology — that is, it can be difficult to recover the most environmentally relevant organisms in culture[20]. For example, in polluted aquifers, in which microorganisms were oxidizing contaminants with the reduction of Fe(III) oxides, there was a significant enrichment in microorganisms with 16S rRNA sequences that were closely related to those of previously cultured *Geobacter* species[22–24]. Coupled with the fact that *Geobacter* species in pure culture are capable of oxidizing organic contaminants with the reduction of Fe(III) oxide[25], this indicated that *Geobacter* species are important in contaminant degradation *in situ*. *Geobacter* species can also remove uranium from contaminated water by

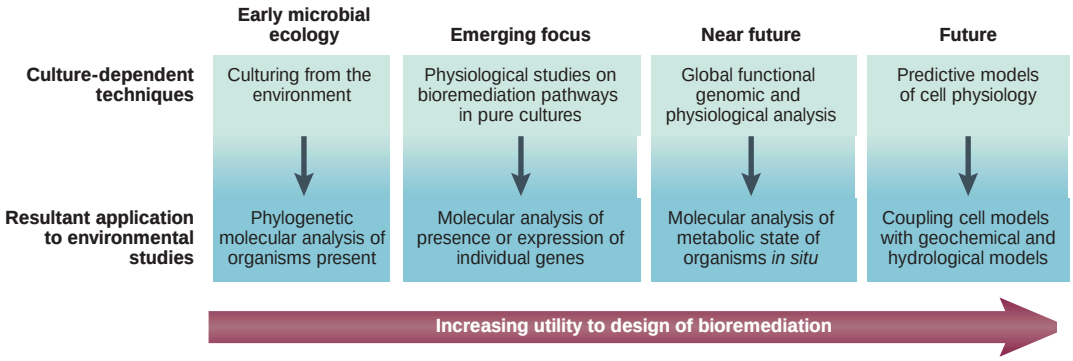

Figure 2 | **Evolution of increasingly sophisticated studies of pure cultures and their application to the study of microbial communities.**

reducing soluble U(VI) to insoluble U(IV)[15]. 16S rRNA sequence analysis showed that, when acetate was added to uranium-contaminated groundwater to promote microbial reduction of U(VI), the number of *Geobacter* species increased by several orders of magnitude, accounting for as much as 85% of the microbial community in the groundwater[16,26]. In aquifers in which the indigenous microbial community was degrading the solvent trichloroethene (TCE), 16S rRNA sequences that are ~99% identical to the 16S rRNA sequence of a pure culture of the TCE-degrader *Dehalococcoides ethanogenes*, were detected[27–29]. Marine sediments with high rates of anaerobic naphthalene degradation were found to be specifically enriched in microorganisms with 16S rRNA sequences closely related to NaphS2, an anaerobic naphthalene degrader that is available in pure culture[30]. There was a close correspondence between the potential for aerobic degradation of the fuel oxygenate methyl *tert*-butyl ether (MTBE) in groundwater and the number of organisms with 16S rRNA sequences that had more than 99% similarity to the MTBE-degrading organism, strain PM-1, which is available in pure culture[31].

The primary limitation of the 16S rRNA technique is that knowledge of the phylogeny of the organisms associated with bioremediation does not necessarily predict important aspects of their physiology[32,33]. For example, microorganisms with 16S rRNA sequences closely related to the TCE-degrader *D. ethanogenes* can differ in the chlorinated compounds that they can degrade[34,35], and predicting which of these compounds an uncultured organism will degrade might not be apparent from analysis of its 16S rRNA sequence alone[29]. Predicting physiology from phylogeny is even more difficult if there are no closely related organisms available in pure culture.

Analysis of genes involved in bioremediation: what can the microorganisms do? Examining the presence and expression of the key genes involved in bioremediation can yield more information on microbial processes than analysis of 16S rRNA sequences[18]. In general, there is a positive correlation between the relative abundance of the genes involved in bioremediation and the potential for contaminant degradation[18,36].

However, the genes for bioremediation can be present but not expressed. Therefore, there has been an increased emphasis on quantifying the levels of mRNA for key bioremediation genes. Often, increased mRNA concentrations can be, at least qualitatively, associated with higher rates of contaminant degradation[36]. For example, the concentrations of mRNA for *nahA* — a gene involved in aerobic degradation of naphthalene — were positively correlated with rates of naphthalene degradation in hydrocarbon-contaminated soil[37]. The reduction of soluble ionic mercury, Hg(II), to volatile Hg(0), is one mechanism for removing mercury from water; the concentration of mRNA for *merA* — a gene involved in Hg(II) reduction — was highest in mercury-contaminated waters with the highest rates of Hg(II) reduction[38]. However, the concentration of *merA* was not always proportional to the rate of Hg(II) reduction[38,39], illustrating that factors other than gene transcription can control the rates of bioremediation processes.

Highly sensitive methods that can detect mRNA for key bioremediation genes in single cells are now available[40]. This technique, coupled with 16S rRNA probing of the same environmental samples, could provide data on which phylogenetic groups of organisms are expressing the genes of interest.

Analysis of the mRNA concentrations for genes other than those directly involved in bioremediation might yield additional insights into the factors that control the rate and extent of bioremediation. Sub-optimal nutrient levels, pH, salinity and other environmental factors can limit the growth and metabolism of organisms that are involved in bioremediation in contaminated environments. Ecological studies of phytoplankton use molecular techniques to evaluate the stress response of photosynthetic microorganisms in the environment[41]. In a similar manner, evaluation of the metabolic state of bioremediating microorganisms through analysis of the mRNA concentrations for key genes that are involved in responding to stress could help to identify modifications to contaminated environments that might promote bioremediation.

Brave new world — the application of genomics

Although the molecular techniques I have outlined have helped to improve our understanding of bioremediation, investigations in this field are on the cusp of a new era which promises — for the first time — to provide a

CHELATOR
A compound that binds iron and other metals and holds them in solution.

ELECTRON SHUTTLE
A compound that accepts electrons from a microorganism and transfers them to an electron-accepting compound, such as Fe(III) oxide.

global insight into the metabolic potential and activity of microorganisms living in contaminated environments. This is the 'genomics era' of bioremediation. With the application of genome-enabled techniques to the study of not only pure cultures, but also environmental samples, it will be possible to develop the models that are needed to model microbial activity predictively under various bioremediation strategies (FIG. 3).

Genome-enabled studies of pure cultures. The application of genomics to bioremediation initially revolutionized the study of pure cultures, which serve as models for important bioremediation processes[42]. Complete, or nearly complete, genome sequences are now available for several organisms that are important in bioremediation (TABLE 1).

Whole-genome sequencing is especially helpful in promoting the understanding of bioremediation-relevant microorganisms, whose physiology has not previously been studied in detail. For example, as noted earlier, molecular analyses have indicated that *Geobacter* species are important in the bioremediation of organic and metal contaminants in subsurface environments. The sequencing of several genomes of microorganisms of the genus *Geobacter*, as well as closely related organisms, has significantly altered the concept of how *Geobacter* species function in contaminated subsurface environments. For instance, before the sequencing of the *Geobacter* genomes, *Geobacter* species were thought to be non-motile, but genes encoding flagella were subsequently discovered in the *Geobacter* genomes[43]. Further investigations revealed that *Geobacter metallireducens* specifically produces flagella only when the organism is growing on insoluble Fe(III) or Mn(IV) oxides (FIG. 4). Genes for chemotaxis were also evident in the *Geobacter* genomes, and experimental investigations have revealed that *G. metallireducens* has a novel chemotaxis to Fe(II), which could help guide it to Fe(III) oxides under anaerobic conditions. Pili genes are present and are also specifically expressed during growth on insoluble oxides[43]. Genetic studies have indicated that the role of the pili is to aid in attachment to Fe(III) oxides, as well as facilitating movement along sediment particles in search of Fe(III) (Mehta, T. *et al.*, manuscript in preparation).

This energy-efficient mechanism for locating and reducing Fe(III) oxides in *Geobacter* species contrasts with the strategies for Fe(III) reduction in other well-studied organisms, such as *Shewanella* and *Geothrix* species. These other organisms release Fe(III) CHELATORS, which solubilize Fe(III) from Fe(III) oxides[44,45], and electron shuttling compounds, which accept electrons from the cell surface and then reduce Fe(III) oxides[44–46]. These strategies make it possible for *Shewanella* and *Geothrix* species to reduce Fe(III) without directly contacting the Fe(III) oxide. However, the synthesis of chelators and ELECTRON SHUTTLES requires a significant amount of energy, and the lower metabolic energy requirements of the *Geobacter* approach is the probable explanation for the fact that *Geobacter* species consistently outcompete other Fe(III)-reducing microorganisms in several subsurface environments[43]. Understanding this, and numerous other previously unsuspected physiological characteristics of *Geobacter* species, is important in guiding the manipulation of conditions in subsurface environments to optimize the ability of *Geobacter* species to remove organic and metal contaminants from polluted groundwater.

The study of the physiology of other microorganisms with bioremediation potential, the genomes of which have been sequenced, is now accelerating in a similar manner. With the completed genome sequences, it is possible — using whole-genome DNA microarrays — to analyse the expression of all the genes in each genome under various environmental conditions. Using proteomic techniques, it is possible to identify which proteins are expressed[42]. Such genome-wide expression analysis provides important data for identifying regulatory circuits in these organisms[47]. This is significant as the mechanisms that control the regulation of the catabolic and respiratory genes that are the most important in bioremediation are largely unknown.

As genetic systems for these environmentally significant organisms become available, it is possible to elucidate the function of the many genes of previously unknown function and to decipher bioremediation pathways. For example, the availability of the *Geobacter* genomes and a genetic system for these organisms is leading to the elucidation of which of the more than 100 *c*-type cytochromes that are apparent in the genome are important in electron transfer to metals[48,49].

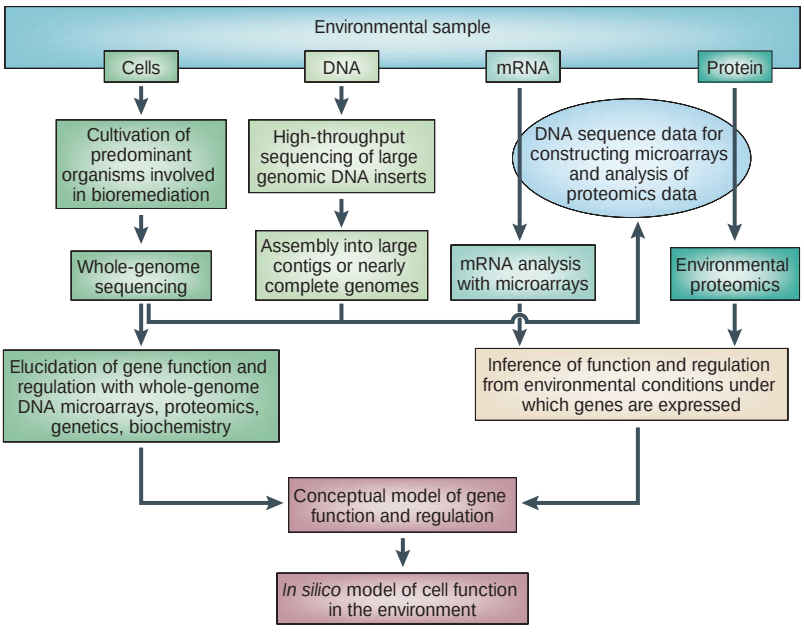

Figure 3 | **Genome-enabled techniques contribute to the development of models of how microorganisms function in contaminated environments.** Cells isolated from the environment provide the opportunity for in-depth physiological analysis as well as information on gene composition that can be used for the analysis of mRNA and proteins that are extracted directly from the environment. Genomic DNA extracted from the environment furnishes data on the genetic potential of as-yet-uncultured organisms. mRNA and proteins extracted from the environment provide information on gene expression under different environmental conditions. Analysis and comparison of pure cultures and mixed communities yields data for the development of models of microbial function in the environment.

Table 1 | Examples of genomes available for microorganisms relevant to bioremediation

Microorganism	Web site for genome documentation	Relevance to bioremediation	References
Dehalococcoides ethanogenes	http://www.tigr.org	Reductive dechlorination of chlorinated solvents to ethylene. The 16S rRNA gene sequence of *D. ethanogenes* is closely related to sequences that are enriched in subsurface environments in which chlorinated solvents are being degraded (see text).	87
Geobacter sulfurreducens, *Geobacter metallireducens*	http://www.tigr.org http://www.jgi.doe.gov	Anaerobic oxidation of aromatic hydrocarbons and reductive precipitation of uranium. 16S rRNA gene sequences closely related to known *Geobacter* species predominate during anaerobic *in situ* bioremediation of aromatic hydrocarbons and uranium.	15,25
Rhodopseudomonas palustris	http://www.jgi.doe.gov	Main organism for elucidating pathways of anaerobic metabolism of aromatic compounds, and regulation of this metabolism.	88
Pseudomonas putida	http://www.tigr.org	Metabolically versatile microorganism capable of aerobically degrading a wide variety of organic contaminants. Excellent organism for genetic engineering of bioremediation capabilities.	89
Dechloromonas aromatica	http://www.jgi.doe.gov	Representative of ubiquitous genus of perchlorate-reducing microorganisms and capable of the anaerobic oxidation of benzene coupled to nitrate reduction.	90,91
Desulfitobacterium hafniense	http://www.jgi.doe.gov	Reductive dechlorination of chlorinated solvents and phenols. *Desulfitobacterium* species are widespread in a variety of environments.	92
Desulfovibrio vulgaris	http://www.tigr.org	Shown to reductively precipitate uranium and chromium. An actual role in contaminated environments is yet to be demonstrated.	93,94
Shewanella oneidensis	http://www.tigr.org	A closely related *Shewanella* species was found to reduce $U_{(VI)}$ to $U_{(IV)}$ in culture, but *Shewanella* species have not been shown to be important in metal reduction in any sedimentary environments.	15
Deinococcus radiodurans	http://www.tigr.org	Highly resistant to radiation and so might be genetically engineered for bioremediation of highly radioactive environments.	95

The range of contaminants that *Dehalococcoides* species can reductively dechlorinate continues to increase, which is consistent with the discovery of at least 15 dehalogenase genes in the genome[35]. It seems likely that analysis of the function of these and other genes in *Dehalococcoides* could greatly enhance our understanding, not only of the diversity of chlorinated contaminants that this organism might bioremediate, but also the factors that regulate its growth and rate of dechlorination in contaminated environments.

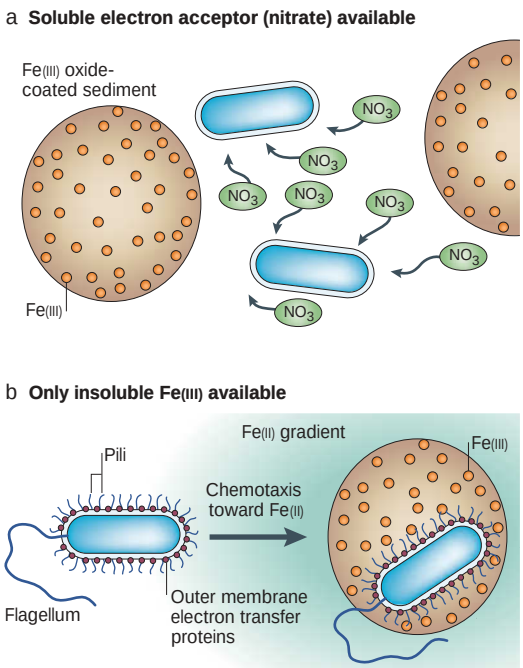

Figure 4 | **Genome-derived model for physiological differences in *Geobacter* during growth on soluble electron acceptors or insoluble $Fe_{(III)}$ oxide.**

In silico *biology*. The genomic approach described above greatly enhances our understanding of the individual physiological capabilities of microorganisms. However, to predict the functioning of an organism in a complex environment, it is necessary to have a more holistic view of metabolism in models that can describe the outcome of the thousands of individual reactions that are simultaneously taking place in a microbial cell. Such descriptions are becoming possible owing to important advances in the development of *in silico* models of cell metabolism[50–53]. Of particular interest is the constraints-based approach, which incorporates the potential metabolic reactions that are possible in an organism, as predicted from the annotated genome, and then, within the bounds of thermodynamic possibilities, carefully accounts for, and balances, the fluxes of metabolic inputs and outputs of all these reactions to describe the overall pathway and output of cell metabolism under a given set of environmental conditions[51]. This yields a steady-state prediction of the boundaries of the metabolic networks of the cell. Detailed kinetic parameters controlling individual enzymatic reactions are generally not included, owing to the paucity of such data and a lack of methods for estimating these parameters from gene sequences. Rather, it is assumed that the cell optimizes the network of cellular reactions to synthesize all of the cellular components that are needed for growth and survival to achieve a particular goal — often defined as the maximum growth rate[54,55]. In instances in which the transcriptional regulation of gene expression is understood

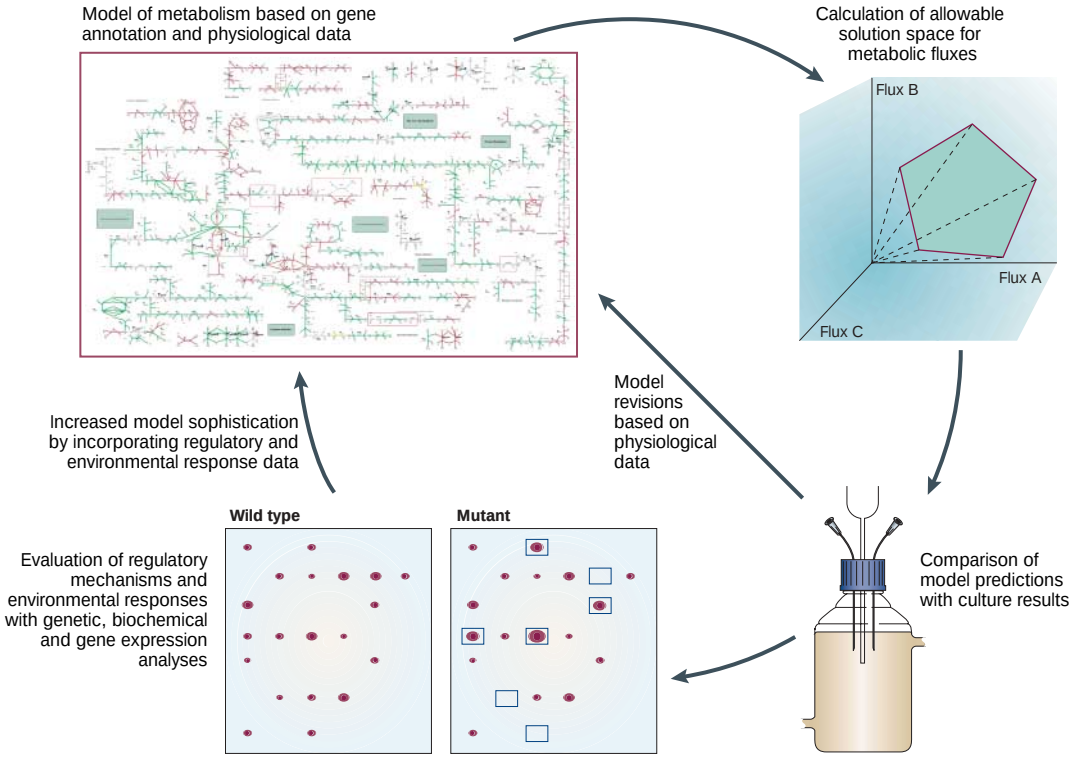

Figure 5 | **Iterative process for the development of *in silico* models for microbial metabolism.**

from experimental data, this can be added as an additional constraint[56]. Alternatively, it might be possible to predict some aspects of regulation of gene expression from the structure of metabolic networks alone[52].

This systems approach to microbial physiology has the ability to predict the metabolic response of organisms to various environmental conditions without the need for information on kinetic parameters for each of the individual reactions that are involved in the pathway[51,52,57]. Substrates that can be metabolized and the nutrients that are required from the environment to support growth can be successfully predicted, as can growth rates under various conditions[55]. Furthermore, modelling in this manner can identify metabolic pathways that need further experimental investigation when, for example, genes required to complete key steps in metabolism seem to be missing from the genome annotation[51]. Iterative modelling and experimental investigations, including predictions and investigations of key mutations[57], can greatly enhance the elucidation of the physiology of a particular microorganism (FIG. 5).

So far, such prokaryotic models have been limited primarily to *Escherichia coli* and pathogens[51–53,55,58]. However, the same modelling approaches should be able to predict contaminant bioremediation by microorganisms that are known to predominate in polluted environments. Such modelling might be most tractable when one type of microorganism is responsible for the bioremediation processes, but the flux-balance approach can also be applied to processes that require the interaction of multiple species[59]. Modelling growth and metabolism under relevant environmental conditions could provide an insight into the factors that might be limiting the rate and extent of bioremediation processes at contaminated sites. The impact of modifying the environment by altering the concentrations of substrates, nutrients or electron acceptors could be modelled — quickly reducing several options down to the most promising alterations for stimulating bioremediation, before conducting laborious and expensive field trials.

Environmental genomics: BAC to the future. It could soon also be possible to apply many of the same genome-enabled techniques that are being used in the study of pure cultures to the study of mixed communities in contaminated environments[60]. For example, genomic DNA extracted from environmental samples can be cloned into large (~40–100 kb) cloning vectors, such as BACTERIAL ARTIFICIAL CHROMOSOMES (BACs), to make a library of environmental genomic DNA[61,62]. This allows evaluation of the genotype of microorganisms that are native to the environment under study, and can be especially informative about groups of microorganisms for which, as yet, there are not any pure-culture isolates. The pioneering studies of DeLong and colleagues in this area are illustrative of the previous applications of this technique. For example, the sequencing of a 130-kb BAC clone — that had been identified as belonging to an abundant, but as-yet-uncultured marine bacterium from its 16S rRNA gene — revealed a gene for rhodopsin, which had previously been observed in Archaea but not Bacteria[63]. Expression of the gene in *E. coli* showed that it coded for

BACTERIAL ARTIFICIAL CHROMOSOME
A vector that can stably maintain a large foreign DNA insert and that can be propagated in *E. coli*.

a light-driven proton pump functioning like the rhodopsin in Archaea[63]. Subsequent field studies demonstrated the significance of this new form of PHOTOTROPHY in the ocean[64]. These important findings, which are based on the sequencing of just one short span of environmental genomic DNA, demonstrate the power of environmental genomics to elucidate novel microbial processes. This approach has obvious applications for evaluating the genotype of microorganisms living in contaminated environments.

However, if environmental genomic approaches are to have a real impact on optimizing bioremediation strategies, studies will need to advance beyond the sequencing of a few isolated segments of genomic DNA. Such studies can provide relatively limited information on the physiology of the organisms being investigated, as is apparent from some studies on single BAC clones that have been published so far[65–67].

The steadily decreasing cost and increasing speed of DNA sequencing has now made it feasible to consider sequencing billions of bases of environmental genomic DNA. This offers the possibility of assembling environmental sequences into complete, or nearly complete, genomes of the individual microorganisms that predominate in the environments of interest, especially in some instances of bioremediation in which a particular genus of microorganism predominates[60]. From such environmental genomic data, it should be possible to produce environmental DNA microarrays, which could be used to analyse the expression of thousands of genes in a particular environment simultaneously (see REF. 68 for proof of concept). Parallel environmental proteomics studies are also imaginable[60]. A global analysis of which genes are being expressed under various conditions in contaminated environments will reveal the metabolic status of the microorganisms and indicate environmental modifications, possibly as simple as the addition of a trace nutrient, which might accelerate the bioremediation. Functional genomic and expression data could be incorporated into *in silico* models that can predict not only the activity of the individual microorganisms responsible for important bioremediation reactions, but also the interactions of these organisms with other microorganisms in the community. In addition to satisfying many of the practical concerns for modelling bioremediation, such studies should yield a basic understanding of the diversity, function, evolution and ecological interactions of microbial communities[61,69], and could go as far as to help answer the age-old question in microbiology of what defines a microbial species[69].

Back to the pure culture

In the end, the application of environmental genomics to bioremediation is bringing us back to the study of pure cultures and basic microbial physiology. In initial studies, the function and regulation of most of the genes documented in environmental genomic investigations will not be known. Although it might be possible to elucidate the function of some genes extracted from the environment by expressing them in *E. coli*[61,62,70], this strategy will only be applicable to screening for known activities that only require a single gene or small operons[71] that can be properly expressed in the host. To elucidate the function of most genes recovered from the environment, it will be necessary to recover the relevant organisms and study gene function in pure culture.

Pure-culture microbiology fell into disfavour among many microbial ecologists over the past two decades as an irrational exuberance about the power of environmental molecular analyses took hold[72]. However, the need for detailed pure-culture studies is now clear. For example, without information on the physiology of closely related pure cultures with known physiologies, descriptions of the distribution of 16S rRNA sequences in the environment quickly deteriorate into the phylogenetic equivalent of stamp collecting. In a similar manner, the interpretation of genotype and expression data from environmental genomic studies requires information on gene function and regulation, which, for the foreseeable future, will only be tractable with pure cultures.

Although the difficulty in culturing environmentally relevant organisms is often bemoaned, culturing these organisms should be possible with a little ingenuity. After all, as previously noted[20], nature can culture all known organisms. With a little thought, humans can probably culture many of these organisms as well. As mentioned earlier, microorganisms closely related to those that predominate in some contaminated environments are already available in culture[3], and the careful replication of environmental conditions during isolation will probably yield more. For example, supplementing seawater with low levels of nutrients enabled the cultivation of members of the SAR11 clade — microorganisms that typically comprise about one-fourth of the marine microbial community, but the presence of which had only previously been detected from 16S rRNA sequences[73]. Simply providing the polymer xylan, rather than monomeric carbon substrates, and extending the incubation time allowed the isolation of important, previously uncultured organisms from soil[74]. Supplementing isolation media with compounds, such as cyclic AMP, that are not substrates but aid in recovery from stressful environmental conditions, or provide a signal that indicates the environment is suitable for growth, could also be helpful[75]. Culturing microorganisms directly in the environment[76], or reproducing environmental gradients in the laboratory[77], are other options. This search for previously uncultured organisms can be greatly accelerated with high-throughput culturing and screening strategies[73,78,79]. The fact that closely related strains of microorganisms can differ significantly in genotype[67,69,80,81] emphasizes the necessity for these pure-culture studies to focus on organisms that have a gene content and organization that is similar to those organisms that are most highly involved in bioremediation *in situ*.

PHOTOTROPHY
A process that involves the gain of energy from light.

Conclusions

The application of genome-enabled techniques to the study of bioremediation is clearly in its infancy. There are many technical issues that will need to be addressed before some of the more novel approaches, such as environmental genome sequencing and arrays, or *in silico* modelling of multi-species consortia, can be routinely employed in this field. However, for bioremediation to advance as a science, there is a strong need for the comprehensive understanding of physiological properties that genome-enabled approaches can provide. The ultimate goal of such studies is the coupling of models of microbial growth and metabolism in contaminated environments with existing geochemical and hydrological models[82] to predict accurately the microbially assisted natural attenuation of contaminants or the likely outcome of engineered strategies to accelerate bioremediation.

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